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Toxicity of Ammonia to Early Life Stages of the Green Sunfish (Lepomis cyanellus)

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16. ABSTRACT

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Pre-hatching embryological stages were found to be the most resistant stages studied. Hatching success was unaffected when incubation was at ammonia concentrations ranging from undetectable through 0.91 mg litre^{-1} NH_3, the highest concentration tested. Growth was inhibited at 0.49 mg litre^{-1} NH_3 but not at 0.22 mg litre^{-1} NH_3. All larvae died within 10 days of hatching at 1.02 mg litre^{-1} NH_3. The 96-h LC50 concentrations at pH values of 6.6, 7.2, 7.7 and 8.7 were 0.50, 1.06, 1.34, and 1.73 mg litre^{-1} NH_3, or 272, 139, 57 and 9 mg litre^{-1} NH_3-N, respectively.

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Toxicity of Ammonia to Early Life Stages of the Green Sunfish *Lepomis cyanellus*

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ABSTRACT

Toxicity of ammonia to early life stages of green sunfish Lepomis cyanellus *was studied. One chronic and four acute exposure studies were conducted. During the chronic study green sunfish were exposed to a series of ammonia concentrations from the blastula stage through 40 days post-hatching. During the acute studies, juvenile green sunfish were exposed at four pH levels, 6·6, 7·2, 7·7 and 8·7, to derive 3-, 6-, 12-, 24-, 48-, 72- and 96-h LC_{50} and EC_{50} concentrations of un-ionised (NH_3) and total ammonia (NH_3–N).*

Pre-hatching embryological stages were found to be the most resistant stages studied. Hatching success was unaffected when incubation was at ammonia concentrations ranging from undetectable through 0·91 mg litre^{-1} NH_3, the highest concentration tested. Growth was inhibited at 0·49 mg litre^{-1} NH_3 but not at 0·22 mg litre^{-1} NH_3. All larvae died within 10 days of hatching at 1·02 mg litre^{-1} NH_3. The 96-h LC_{50} concentrations at pH values of 6·6, 7·2, 7·7 and 8·7 were 0·50, 1·06, 1·34 and 1·73 mg litre^{-1} NH_3, or 272, 139, 57 and 9 mg litre^{-1} NH_3–N, respectively.

INTRODUCTION

The necessity to base environmental control regulations on scientifically defencible experimentation prompted the following study to determine the responses of green sunfish *Lepomis cyanellus* to ammonia. Considerable information on the sensitivity of cold-water fishes to ammonia

has already been reported (US Environmental Protection Agency, 1977; Willingham *et al.*, 1979; Alabaster & Lloyd, 1980), but available knowledge of the responses of warm-water fishes was limited, particularly for effects of partial chronic exposures and of exposures under different pH conditions. Therefore, green sunfish eggs, larvae and juveniles were exposed to a series of ammonia concentrations to determine sublethal and lethal response concentrations.

The study was conducted in two phases: the first was initiated with newly fertilised eggs and extended through 40 days after hatching. During the second phase, juveniles were exposed to a series of ammonia concentrations for a maximum of 96 h at four pH levels. These studies provided an indication of the relationship between short- and long-term sensitivity of green sunfish to ammonia. The usefulness of this acute-to-chronic toxicity ratio lies in estimating chronic effects (at similar pH values) when only acute responses are known, or can be determined in the time available (US Environmental protection Agency, 1980).

Ammonia concentrations are expressed as un-ionised ammonia (NH_3) throughout, since it is generally proposed that it is the molecular form of ammonia that is primarily responsible for the adverse effects of ammonia on aquatic life (US Environmental Protection Agency, 1977; Willingham *et al.*, 1979). However, total ammonia concentrations were also reported so that the relationship between total and un-ionised ammonia toxicity could be known and compared. We also studied the influence of the pH of the exposure water on the responses elicited. These tests were conducted since the percentage of un-ionised ammonia and its toxicity to aquatic organisms is strongly altered by the pH of the exposure water (Thurston *et al.*, 1981*a*).

Previous toxicological studies of ammonia toxicity to green sunfish are limited to acute exposure responses (Jude, 1973; Reinbold & Pescitelli, 1982). Jude (1973) reported ammonia toxicity results expressed as total N at pH 7·8 and 12·3 °C. It can be calculated from Jude's data that the 96-h LC_{50} for green sunfish was 0·61 mg litre^{-1} as un-ionised ammonia. Reinbold & Pescitelli (1982) reported a 96-h LC_{50} of 1·08 mg litre^{-1} as un-ionised ammonia when the pH was 8·1–8·5 and the temperature 26·2 °C. To our knowledge, no studies of ammonia toxicity to green sunfish have been conducted to provide chronic exposure information.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the US Environmental Protection Agency.

MATERIALS AND METHODS

Chronic test

Green sunfish collected in northeastern Wisconsin were brought to our laboratory where they were allowed to spawn in a 188-litre aquarium at 22 °C. Embryos still in early cleavage stages were collected, counted by units of five into experimental lots of 30 and placed in incubation chambers. These experimental lots were then randomly transferred to the testing apparatus, two chambers per duplicate treatment level. Transfer to the test apparatus was completed while the embryos were still in the high blastula stage. Incubation was at a mean temperature of $22{\cdot}0 \pm 0{\cdot}1$ °C, a dissolved oxygen concentration of $8{\cdot}0 \pm 0{\cdot}1$ mg litre^{-1} (>85% saturated) and a pH of $8{\cdot}0 \pm 0{\cdot}1$ ($\pm$ values are 95% confidence limits).

The experimental design for the incubation study included six treatments in duplicate: a control (with no artificial addition of ammonia) and five ammonia concentrations. Total ammonia concentrations were determined for one replicate from each of the six treatment levels at least two times per week. Ammonia-nitrogen was determined by the Nesslerization method (method 417B, American Public Health Association *et al.*, 1981). Nitrate and nitrite were determined by American Public Health Association *et al.* (1981) methods 418A and 419, respectively. Corresponding pH and temperature measurements were used to calculate unionised ammonia (NH_3) concentrations from the table of Thurston *et al.* (1979). A paired comparisons (*t*-test) of simultaneously tested ammonia levels between replicate tanks at the highest treatment level showed no statistical difference ($P < 0{\cdot}05$), between the replicates, the difference between the means was less than 2%.

Treatment concentrations were achieved using a continuous flow type diluter (Benoit *et al.*, 1982). Ammonia was added to the test apparatus by pumping a concentrated ammonium chloride (Fisher certified ACS grade) solution prepared in distilled water. The ammonia stock solution pH was adjusted to that of Lake Superior dilution water by addition of sodium hydroxide. Water quality characteristics of the unaltered Lake Superior dilution water were assumed to be similar to that reported by Biesinger & Christensen (1972). Mean experiment wide water quality characteristics, of non-experimental treatment affected parameters, measured during the four days of incubation and the following 40 days of the growth study were as follows: pH $7{\cdot}9 \pm 0{\cdot}1$ ($N = 514$), dissolved

TABLE 1
Mean Nitrite Concentrations[a] at Respective Ammonia Treatment Concentrations During Chronic Exposure Study

	Mean un-ionised ammonia concentration (mg litre^{-1} NH_3)					
	Control	*0·05*	*0·12*	*0·25*	*0·48*	*0·91*
Nitrite (μg litre^{-1} as NO_2–N)	1·9 ± 0·3	32·4 ± 15·4	36·6 ± 13·6	56·9 ± 17·4	64·1 ± 21·7	92·2 ± 25·2

[a] Sample size was 13 for all treatments except at 0·91 mg litre^{-1} un-ionised ammonia where it was 28.

oxygen 7·9 ± 0·1 mg litre^{-1} (92% saturation) ($N = 42$), temperature 22·0 ± 0·1 °C ($N = 487$), total alkalinity 43·0 ± 0·4 mg litre^{-1} as $CaCO_3$ ($N = 56$), total hardness 42·8 ± 0·4 mg litre^{-1} as $CaCO_3$ ($N = 54$), and nitrate (NO_3–N) 0·27 ± 0·02 mg litre^{-1} ($N = 42$). Nitrite values in the test chambers varied with the ammonia concentrations (Table 1).

Embryo incubation chambers were glass jars with stainless steel screen bottoms, elevated on 1·5-cm legs to ensure water circulation around the eggs (McCormick, 1978). Water flow was approximately 100 ml min^{-1} through the 4-litre (10 × 40 × 10 cm deep) water baths containing 3 litres of water each. Circulation around the eggs was ensured by self-starting siphons in the outlet of each water bath which caused the test solution levels to fluctuate, and to enter and discharge through the screen bottoms of the embryo exposure chambers. Each day the embryos were examined under a dissecting microscope to differentiate between normal and obviously deformed larvae. Incubation was completed in 3–4 days at which time only normal larvae were reported as hatching successfully.

Four days after the last embryos hatched, the larvae were combined and released into a 1·5 litre (10 × 15 × 10·5 cm deep) growth chamber containing 1·1 litres of test solution. Water flow through these chambers was the same as during incubation. Following their transfer to the smaller growth chambers, the larvae were fed for the first time. They remained in these growth chambers for 10 days, and then were transferred to 4-litre rearing chambers. The interim chambers were used to facilitate cleaning and observation during the critical first feeding period when food density is critical and the larvae were demersal and difficult to see. Feeding was to excess, five times per day, except on weekends when the number of feedings was reduced to 3–4 times per day. Initial feedings were with newly

hatched San Francisco Bay brand brine shrimp *Artemia* sp. nauplii since these tend to be smaller than those from some other sources. Later, after the young fish had made the transition to exogenous feeding, nauplii produced from cysts from the Great Salt Lake in Utah were used. On the 40th day, and 24 h after last feeding, they were sacrificed in an ice bath, blotted dry and individually weighed to the nearest milligram.

Because of complete mortality within 10 days of hatching in both replicates at 1·02 mg litre^{-1} NH_3, a second set of two replicates of 60 fish each was placed in that concentration and the growth studied as though part of the original samples. These fish were from the same embryo source as the originals, but had been raised in a separate stock culture on the same feed and water source as the controls. All these larvae had made the transition to exogenous feeding before exposure.

Testing for statistical differences in responses, due to the ammonia exposures, was by analysis of variance. The difference between growth at different ammonia concentrations, significant at the 95% level of confidence, was determined by Bonferroni's multiple pairwise comparisons (Neter & Wasserman, 1974).

Acute tests

Juvenile green sunfish hatched and reared at 22 °C from the same lot of eggs used in the chronic growth study were used in the acute toxicity studies. The fish were fed brine shrimp nauplii until 24 h before acute ammonia toxicity testing. At the time of distribution to the exposure tanks, fish from a subsample had a mean weight of 62·5 ± 10·6 mg per fish. The test fish were distributed to the exposure chambers in a stratified random manner, 20 fish per exposure chamber. No food was provided during the acute toxicity tests.

The acute test exposure chambers had a volume of 2 litres (9·5 × 19·5 × 10·5 cm deep) and contained 1 litre of test solution. The water flow to each chamber was 40 ml min^{-1}. The mean experiment wide test temperature, dissolved oxygen and total hardness level in the exposure chambers were 22·4 ± 0·1 °C ($N = 210$), 8·1 ± 0·1 mg litre^{-1} (92% saturated) ($N = 11$) and 43·1 ± 0·4 mg litre^{-1} as $CaCO_3$ ($N = 12$), respectively. Total alkalinity (in mg litre^{-1} $CaCO_3$) varied with the pH of the test exposures as follows: pH 6·6 exposures, mean alkalinity was 9·8 ± 0·5; pH 7·2 exposures, mean alkalinity was 27·2 ± 0·7; pH 7·7 exposures, mean alkalinity was 45·0 ± 1·9; pH 8·7 exposures, mean

alkalinity was 49·8 ± 3·6. In each case the mean is of single analyses, one taken in each of seven test exposure chambers. Nitrite levels ranged between 0·6 and 1·3 μg litre^{-1} as NO_2–N and nitrates were at 0·3 mg litre^{-1} as NO_3–N or lower across all ammonia and pH concentrations (N = once each per treatment).

Ammonia was added to the dilution water, mixed and proportioned for delivery to the exposure chambers by a continuous flow diluter similar to that used in the chronic exposure study. Seven treatments were provided at each of four pH regimes, two replicates per treatment. Treatments were a combination of pH and ammonia concentration. The ammonium chloride stock solutions were prepared in the same manner as for the chronic test exposures; pH of the ammonia stock solution was adjusted by addition of sodium hydroxide or hydrochloric acid to attain the desired test pH.

Dilution water to the test chambers was supplied from two 25-litre stainless steel head tanks. The water in each head tank was pumped over a vertical cylindrical screen to remove excess dissolved gases, including CO_2 liberated by addition of acid for pH adjustment. The pH of the dilution water in one tank was unadjusted (pH 7·7) while that in a second tank was held at about 6·6 by continuous addition of dilute hydrochloric acid. By mixing water from both head tanks with glass–Teflon mixing valves, a third test water of pH 7·2 was obtained. By adding a dilute solution of sodium hydroxide directly to the ambient head tank water (pH 7·7) before it entered the diluter, a fourth dilution water of pH 8·7 was provided. Assuming that this gas was at equilibrium with air consequent to the degassing procedure described, free CO_2 concentrations were calculated to be 4·9 mg litre^{-1} at pH 6·6, 3·4 mg litre^{-1} at pH 7·2, 1·8 mg litre^{-1} at pH 7·7 and 0·2 mg litre^{-1} at pH 8·7 (American Public Health Association *et al.*, 1981, Method 406 C). All these concentrations were safely within the tolerance range for rainbow trout *Salmo gairdneri* (Lloyd & Jordan, 1964) and thus probably also safe for green sunfish.

Mortalities among the exposed fish were recorded at 3, 6, 12, 24, 48, 72 and 96 h after onset of exposure to the toxicant. Loss of equilibrium was recorded at 24, 48, 72 and 96 h of exposure. The LC_{50} and EC_{50} ammonia concentrations at each exposure time and test pH were calculated using the Trimmed Spearman–Karber Method (Hamilton *et al.*, 1977). The EC_{50} values were calculated using the summation of the fish that had died or were unable to maintain equilibrium at each recorded observation.

Acute-to-chronic ratio

Once both the geometric mean of the maximum no observed effect concentration and the minimum effect concentration and the 96-h LC_{50} had been determined, an acute-to-chronic ratio was calculated. The acute-to-chronic ratio was derived by dividing the acute 96-h LC_{50} concentration by the geometric mean of the minimum adverse effect and maximum no effect concentrations when both were conducted in water of similar pH.

RESULTS

Chronic test

Hatching success was unaffected by exposure to un-ionised ammonia concentrations from control through 0·91 mg litre^{-1} NH_3 ($P > 0·05$). The mean hatching success for all treatments, as measured by numbers of normal live larvae produced, was 73% of the original embryos in the samples (Table 2). However, by 3–4 days post-hatching 87% (46 of 53—one complete replicate was lost due to procedural error) of the surviving larvae in the highest exposure concentration (0·91 mg litre^{-1} NH_3) had developed recognisable deformities. Observed deformities were various

TABLE 2
Hatching Success of Green Sunfish Embryos Incubated in Lots of 30 at Different Concentrations of Un-ionised Ammonia

Replicate exposure chambers	*Duplicate embryo lots*	*Mean un-ionised ammonia concentration (mg litre^{-1} NH_3)*[a]					
		Control	*0·05*	*0·12*	*0·25*	*0·48*	*0·91*
A	I	26	22	26	21	28	22
	II	20	13	28	20	24	11
B	I	19	24	25	19	26	21
	II	23	24	24	20	20	19
Mean A and B		22	21	26	20	24	18

[a] Only one analysis done during this portion of the study.

TABLE 3

Mean Wet Weights (mg) of Juvenile Green Sunfish 44 Days after Fertilisation when Continuously Exposed to Different Un-ionised Ammonia Concentrations at 22 °C

Replicate exposure chambers	*Mean un-ionised ammonia concentrations (mg litre^{-1} NH_3)*					
	<0·01 Control	*0·05 (± <0·01)*[a]	*0·11 (±0·01)*	*0·22 (±0·01)*	*0·49 (±0·03)*	*1·02 (±0·06)*
A	116	143	123	109	85	46[c]
	[23][b]	[16]	[26]	[18]	[6]	[2]
B	114	126	130	114	101	61[c]
	[6]	[22]	[27]	[15]	[18]	[12]
Mean A and B	115[d]	134	126	112	93	54[c]

[a] Values in parentheses = 95% confidence limit.

[b] The values in brackets are numbers of surviving individuals from which the mean weights were determined.

[c] The growth reported here is that of survivors from 60 larvae that were reintroduced to this exposure concentration after total mortality of the original test lots.

[d] Weights underlined by similar lines are part of statistically homogeneous sets at the 95% level of confidence.

degrees of curvature of the spine and/or pericardial oedema. Deformities among the larvae in all lower exposure concentrations were 1–3%.

The 10 days following hatching are a most critical period, when larval fish must make the transition from endogenous to exogenous feeding (Toetz, 1966). Mortalities during this period were extensive and reached 100% in both replicates of the highest ammonia concentration. Once the conversion to feeding on live brine shrimp nauplii was accomplished, mortality rates were reduced but not eliminated (Table 3). These results indicate that the maximum no observed adverse effect concentration, based on growth inhibition, is 0·22 mg litre^{-1} NH_3 and the minimum adverse effect concentration is 0·49 mg litre^{-1} NH_3. The geometric mean of these two values, an estimate of the chronic effect concentration, is 0·33 mg litre^{-1} NH_3. At 1·02 mg litre^{-1} NH_3 complete mortality of all larvae occurred within 10 days of hatching. Even among the second lots of fish introduced into the two replicates of the highest exposure concentration, mortalities continued to occur until termination of the study; the growth of survivors was severely retarded (Table 3).

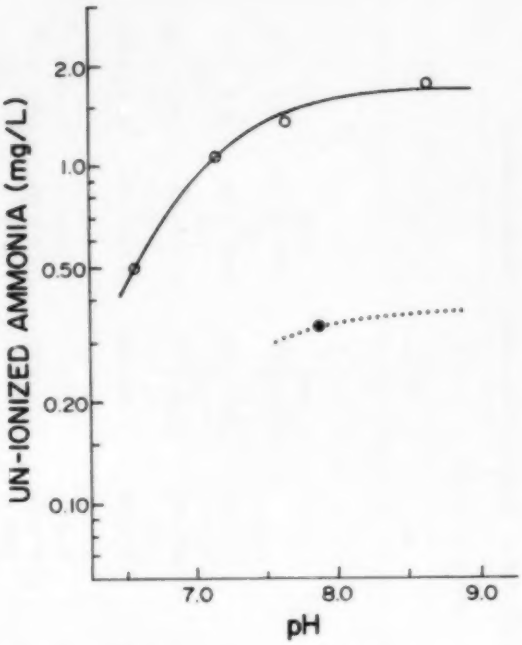

Fig. 1. 96-h LC_{50} values for un-ionised ammonia for juvenile green sunfish at pH values of 6·6, 7·2, 7·7 and 8·7 (—○—). The chronic effect exposure concentration at pH 7·9 (●) and projected effect concentrations at other pH values(.....) are drawn over the pH range where it may be reasonable to assume a relatively constant relationship between acute and chronic values. (The chronic effect concentration was derived from the 44-day growth study, and is the geometric mean of the maximum no effect concentration and the minimum effect concentration (Table 3). Broderius *et al.* (details to be published) found increasing acute-to-chronic ratios as pH decreased below pH 7·8, but the ratios were little affected or unaffected at pH values between 7·8 and 8·7.)

Acute tests

Acute exposures of juvenile green sunfish to a graded series of ammonia concentrations at pH values of 6·6, 7·2, 7·7 and 8·7 provided 96-h LC_{50} values of 0·50, 1·06, 1·34 and 1·73 mg litre^{-1} NH_3, respectively (Table 4). Figure 1 presents these 96-h LC_{50} values as related to their respective test pH values and a curve fitted to the four points. The shape of the curve and its position with respect to pH and NH_3 concentration is described

TABLE 4
LC_{50} and EC_{50} Values for Juvenile Green Sunfish Acutely Exposed to Ammonium Chloride Solutions

pH	*Exposure time (h)*						
	3	*6*	*12*	*24*	*48*	*72*	*96*
Un-ionised ammonia (mg litre^{-1} NH_3)							
LC_{50}							
6·6	—	—	—	—	0·87 (0·81–0·95)[a]	0·68 (0·62–0·75)	0·50 (0·47–0·53)
7·2	1·38 (1·35–1·41)	1·36 (1·32–1·41)	1·28 (1·21–1·36)	1·23 (1·15–1·33)	1·17 (1·06–1·29)	1·09 (0·98–1·21)	1·06 (0·96–1·19)
7·7	1·92 (1·76–2·09)	1·82 (1·67–1·98)	1·71 (1·58–1·85)	1·57 (1·45–1·70)	1·37 (1·29–1·47)	1·34 (1·25–1·43)	1·34 (1·25–1·44)
8·7	1·65 (–)	1·63 (1·59–1·67)	1·61 (1·56–1·66)	1·56 (1·48–1·64)	1·76 (1·65–1·86)	1·74 (1·63–1·85)	1·73 (1·60–1·86)
EC_{50}							
6·6				0·42 (0·38–0·45)	0·64 (0·58–0·70)	0·64 (0·58–0·70)	0·50 (0·47–0·53)
7·2				0·50 (0·46–0·55)	0·54 (0·50–0·58)	0·66 (0·62–0·71)	0·82 (0·74–0·92)
7·7				0·96 (0·88–1·04)	0·88 (0·84–0·93)	0·90 (0·85–0·96)	1·19 (1·09–1·31)
8·7				1·26 (1·16–1·36)	1·18 (1·14–1·22)	1·20 (1·16–1·24)	1·29 (1·20–1·38)

Total ammonia (mg litre^{-1} NH_3–N)							
LC_{50}							
6·6	—	—	—	—	547 (503–596)	395 (357–438)	272 (255–290)
7·2	212 (206–218)	208 (200–217)	195 (182–209)	187 (173–203)	165 (148–184)	144 (128–162)	139 (124–157)
7·7	82 (75–89)	78 (71–84)	73 (67–79)	67 (62–73)	58 (55–63)	57 (53–61)	57 (53–62)
8·7	10 (NC)[b]	9 (9–10)	9 (9–10)	9 (9–10)	10 (9–10)	10 (9–10)	9 (9–10)
EC_{50}							
6·6				248 (227–270)	392 (353–434)	366 (330–406)	272 (255–290)
7·2				74 (69–80)	76 (71–81)	85 (79–91)	107 (95–120)
7·7				40 (37–44)	37 (35–39)	38 (35–40)	50 (46–56)
8·7				7 (7–8)	7 (6–7)	6 (6–7)	7 (6–7)

[a] Values in parentheses are 95% confidence limits.
[b] NC, Not calculable.

by the equation:

$$96\text{-h } LC_{50}\,\text{mg litre}^{-1}\,NH_3 = \frac{1{\cdot}69}{1 + 10^{(6{\cdot}98 - pH)}}$$

This formula enables calculation of 96-h LC_{50} values for interpoint pH values.

The influence of duration of exposure on both the LC_{50} and EC_{50} at various pH values is presented in Table 4. The expected time to onset of loss of equilibrium at different ammonia concentrations can be estimated by reference to Table 4. It was observed, however, that some individuals that lost equilibrium subsequently recovered. There was also a sequence of responses that preceded loss of equilibrium. Hyperactivity was the first behavioural change observed in the ammonia-exposed fish. This frequently progressed to loss of equilibrium and then floating in an inverted position at the surface. Eventually fish so affected sank to the bottom in what seemed to be an anaesthetised state. At the higher ammonia concentrations, these responses ultimately culminated in death. However, when ammonia-free water was reintroduced into the exposure units apparent complete recovery occurred among all affected fish that had not already died.

A more complex sequence of responses occurred in concentrations not high enough to cause a continuous decline in condition and ultimately in death. The response of some fish at these concentrations can be characterised in two ways. First, there was a loss of equilibrium for a time (usually after about 24 h of exposure) followed by complete recovery. Second, recovery might not occur until about 48 h of exposure, then only eventually to end in death.

Acute-to-chronic ratio

The 96-h LC_{50} obtained at pH 7·7 (1·34 mg litre^{-1} NH_3) is related to the chronic effect value (0·33 mg litre^{-1} NH_3) derived in the embryo–larval test since both were obtained using Lake Superior water without pH adjustment. Using these two values, an acute-to-chronic ratio of 4·06 was calculated.

DISCUSSION

Chronic test

When compared with other life stages studied in these tests, embryos of green sunfish were found to be relatively resistant to adverse effects of

ammonia. This is consistent with findings reported by Rice & Bailey (1980) for pink salmon *Oncorhynchus gorbuscha* and by Calamari *et al.* (1981) for rainbow trout *Salmo gairdneri*. Hatching success of green sunfish was not affected at ammonia concentrations as high as 0·91 mg litre^{-1} NH_3 ($P > 0{\cdot}05$). Growth was, however, significantly reduced from that of control fish ($P < 0{\cdot}05$) at a concentration between 0·22 and 0·49 mg litre^{-1} NH_3. Particularly sensitive was the 'critical period' (Toetz, 1966) when the larvae first require exogenous food to survive. Rice & Stokes (1975), working with rainbow trout *S. gairdneri*, also reported this to be a life stage particularly sensitive to ammonia.

Growth was the most sensitive measure of chronic effect. Growth finds its value as a measure of effect indicative of environmental quality since it integrates results of many effects on many vital life processes, and it pertains to the concept of fish as a crop to be harvested. An adverse effect of ammonia on growth of juvenile green sunfish was observed at a concentration of 0·49 mg litre^{-1} NH_3 but not at 0·22 mg litre^{-1} NH_3.

It is of interest to note that growth at the two lowest ammonia treatments (0·05 and 0·11 mg litre^{-1} NH_3) was actually elevated ($P < 0{\cdot}05$) above that of the control fish exposed to unaltered Lake Superior water (Table 3). Growth stimulation at low ammonia concentrations is probably a revelation of hormesis mediated via growth-hormone stimulation at stressful concentrations of ammonia too low to be growth-inhibiting (Selye, 1976). Robinson-Wilson & Seim (1975) reported a similar growth stimulation in juvenile chinook salmon *Oncorhynchus tshawytscha* at low (but above background) ammonia concentrations, indicating that the response is not peculiar to green sunfish in Lake Superior water.

Acute tests

The time-dependent effects of acute ammonia exposure are best visualised by reference to Table 4, which provides time-dependent response data (LC_{50} and EC_{50} values) for both un-ionised and total ammonia. These data indicate that loss of equilibrium occurs at a lower concentration of ammonia than does mortality and that far less un-ionised ammonia is required to elicit the same adverse effect than of total ammonia. This is particularly true at the 24- and 48-h exposure periods. Loss of equilibrium can be just as devastating to a green sunfish population as death caused directly by ammonia exposures. This can occur since predation is an ever-present hazard to juvenile fishes in their natural habitats and incapacitated

prey are particularly vulnerable and attractive to competent predators (Mueller, 1968; Hedtke & Norris, 1980).

The greater relative toxicity of un-ionised ammonia to that of total ammonia can best be observed in Table 4 and by comparing LC_{50} or EC_{50} values for the same exposure times and pH values. Less obvious, but equally indicative of these relative toxicities, are the lower total ammonia LC_{50} and EC_{50} values at pH 8·7 than at lower pH values; at higher pH values less of the total ammonia is in the un-ionised form (Emerson *et al.*, 1975). As can be observed in Fig. 1, un-ionised ammonia toxicity increases at lower pH. The effects of pH on un-ionised ammonia toxicity become less important at higher pH values, particularly above pH 8. These findings are in agreement with those of Thurston *et al.* (1981*a*) for rainbow trout and fathead minnows *Pimephales promelas*.

In using the data from our studies to estimate safe exposures it should be kept in mind that temperature has been implicated as having an important influence on ammonia toxicity. Ammonia was reported to be more toxic to bluegills, fathead minnows and rainbow trout at cooler temperatures, particularly below 5 °C (Reinbold & Pescitelli, 1982). All of our studies were at 22 °C. Dissolved oxygen has also been found significantly to alter ammonia toxicity to fishes (Thurston *et al.*, 1981*b* and references cited therein). Ammonia generally exhibits greater toxicity at lower dissolved oxygen levels. Our tests were at relatively high dissolved oxygen levels. Since high ammonia concentrations are frequently associated with organic waste discharges, low dissolved oxygen levels can be expected at many sites where ammonia is of concern. Thus dissolved oxygen as a factor in ammonia toxicity must be given real consideration in estimating responses of fishes to ammonia; this may be particularly true during cold-water periods.

Acute-to-chronic ratio

Acute-to-chronic ratios have been used with some success to estimate chronic exposure concentrations of toxicants when only acute values are known (US Environmental Protection Agency, 1980). However, with ammonia it must be pointed out that the acute-to-chronic ratio is pH-dependent below about pH 7·5 (S. J. Broderius, R. A. Drummond, J. T. Fiandt and C. L. Russom, details to be published). Therefore it is important that the ratio is derived from data within a limited pH range.

CONCLUSIONS

The purpose of generating ammonia toxicity data for this warm-water species (green sunfish) was because the previous studies were conducted predominantly with cold-water species. The green sunfish was, however, found to have a sensitivity to ammonia about equal to the upper range for salmonids and the midrange for nonsalmonids (US Environmental Protection Agency, 1977; Willingham *et al.*, 1979; Alabaster & Lloyd, 1980). The new data from this study are valuable in providing a greater understanding of the sensitivity and responses of green sunfish to ammonia exposure.

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